1600

RAW SEQUENCE LISTING                    DATE: 03/11/2003
PATENT APPLICATION:  **US/09/677,584**       TIME: 10:54:53

Input Set : **A:\PTO.VSK.txt**
Output Set: **N:\CRF4\03112003\I677584.raw**

```
       2 <110> APPLICANT: DIVERSA CORPORATION
       3        SHORT, Jay
W-->   4 <120> TITLE OF INVENTION: WHOLE CELL ENGINEERING BY MUTAGENIZING A SUBSTANTIAL PORTION
OF A
W-->   5        STARTING GENOME, COMBINING MUTATIONS, AND OPTIONALLY REPEATING
       7 <130> FILE REFERENCE: DIVER1510WO-1
C-->   9 <140> CURRENT APPLICATION NUMBER: US/09/677,584
C-->  10 <141> CURRENT FILING DATE: 2000-09-30
      12 <150> PRIOR APPLICATION NUMBER: US 09/677,584
      13 <151> PRIOR FILING DATE: 2000-09-30
      15 <150> PRIOR APPLICATION NUMBER: US 09/594,459
      16 <151> PRIOR FILING DATE: 2000-06-14
      18 <160> NUMBER OF SEQ ID NOS: 33
      20 <170> SOFTWARE: PatentIn version 3.0
      22 <210> SEQ ID NO: 1
      23 <211> LENGTH: 5818
      24 <212> TYPE: DNA
      25 <213> ORGANISM: Escherichia coli
      27 <400> SEQUENCE: 1
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      30 ttccatgtga cctcctaaca tggtaacgtt catgataact tctgctcttc atcgtgcggc    120
      32 cgactgggct aaatctgtgt tctcttcggc ggcgctgggt gatcctcgcc gtactgcccg    180
      34 cttggttaac gtcgccgccc aattggcaaa atattctggt aaatcaataa ccatctcatc    240
      36 agagggtagt gaagccatgc aggaaggcgc ttaccgattt taccgcaatc ccaacgtttc    300
      38 tgccgaggcg atcagaaagg ctggcgccat gcaaacagtc aagttggctc aggagtttcc    360
      40 cgaactgctg gccattgagg acaccacctc tttgagttat cgccaccagg tcgccgaaga    420
      42 gcttggcaag ctgggctcta ttcaggataa atcccgcgga tggtgggttc actccgttct    480
      44 cttgctcgag gccaccacat tccgcaccgt aggattactg catcaggagt ggtggatgcg    540
      46 cccggatgac cctgccgatg cggatgaaaa ggagagtggc aaatggctgg cagcggccgc    600
      48 aactagccgg ttacgcatgg gcagcatgat gagcaacgtg attgcggtct gtgaccgcga    660
      50 agccgatatt catgcttatc tgcaggacag gctggcgcat aacgagcgct tcgtggtgcg    720
      52 ctccaagcac ccacgcaagg acgtagagtc tgggttgtat ctgatcgacc atctgaagaa    780
      54 ccaaccggag ttgggtggct atcagatcag cattccgcaa aagggcgtgg tggataaacg    840
      56 cggtaaacgt aaaaatcgac cagcccgcaa ggcgagcttg agcctgcgca gtgggcgcat    900
      58 cacgctaaaa caggggaata tcacgctcaa cgcggtgctg ccgaggaga ttaacccgcc    960
      60 caagggtgag accccgttga aatggttgtt gctgaccggc gaaccggtcg agtcgctagc   1020
      62 ccaagccttg cgcgtcatcg acatttatac ccatcgctgg cggatcgagg agttccataa   1080
      64 ggcatggaaa accggagcag gagccgagag gcaacgcatg gaggagccgg ataatctgga   1140
      66 gcggatggtc tcgatcctct cgtttgttgc ggtcaggctg ttacagctca gagaaagctt   1200
      68 cacgctgccg caagcactca gggcgcaagg gctgctaaag gaagcggaac acgtagaaag   1260
      70 ccagtccgca gaaacggtgc tgacccccgga tgaatgtcag ctactgggct atctggacaa   1320
      72 gggaaaacgc aagcgcaaag agaaagcagg tagcttgcag tgggcttaca tggcgatagc   1380
      74 tagactgggc ggttttatgg acagcaagcg aaccggaatt gccagctggg cgcccctctg   1440
      76 gtaaggttgg gaagccctgc aaagtaaact ggatggcttt cttgccgcca aggatctgat   1500
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 80 aagatggatt gcacgcaggt tctccggccg cttgggtgga gaggctattc ggctatgact 1620
 82 gggcacaaca gacaatcggc tgctctgatg ccgccgtgtt ccggctgtca gcgcaggggc 1680
 84 gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg caggacgagg 1740
 86 cagcgcggct atcgtggctg gccacgacgg gcgttccttg cgcagctgtg ctcgacgttg 1800
 88 tcactgaagc gggaagggac ·tggctgctat tgggcgaagt gccggggcag gatctcctgt 1860
 90 catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg cggcggctgc 1920
 92 atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc atcgagcgag 1980
 94 cacgtactcg gatggaagcc ggtcttgtcg atcaggatga tctggacgaa gagcatcagg 2040
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104 acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt gacgagttct 2340
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112 gctcgatccc ctcgcgagtt ggttcagctg ctgcctgagg ctggacgacc tcgcggagtt 2580
114 ctaccggcag tgcaaatccg tcggcatcca ggaaaccagc agcggctatc cgcgcatcca 2640
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122 ctcaccaccc actcgagctg gatacttccc gtccgccagg gggacatgcc ggcgatgctg 2880
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126 cagggcgccg cccgagtctt cgcctcggcg gcgggcgctc tgctcatgga gcgcgcgtcc 3000
128 ggggccgggg accttgcaca gatagcgtgg tccggccagg acgacgaggc ttgcaggatc 3060
130 ctctgcgaca ccgccgctcg tctgcacgcg ccgcggtccg gaccgccgcc cgatctccat 3120
132 ccgctacagg aatggttcca gccgcttttc cggttggccg ctgagcacgc ggcacttgcg 3180
134 cccgccgcca gcgtagcgcg ccaacttctg gcggcgccgc gcgaggtgtg cccgctccac 3240
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138 ccgcacggac tgctcggcga gcgcaccttc gactatgcca acatcttcac gaatcccgat 3360
140 ctcagcgacc ccggtcgccc gcttgcgatc ctgccgggca ggctggaggc tcgactcagc 3420
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148 cacctggtcg tcgagctagg tcaggccgtg tcgggcgtga tccgctggaa gtcgttgcgg 3660
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162 gctcgggaac tggcgcaggc cgcgaaggac attgccgccg ataccggcct ggagcatcgc 4080
164 cccgtggccg acggccagcg cgttgccggc gtctaccggc gccccgtcat gctcgccagc 4140
166 gggcgaaatg ggatgcttga tgacgccaag gggtccagcc tcgtgcggtg gaagcccatc 4200
168 aacagcggc ttggggagca gctcgccgcg acggtgcgcg gtggcggcgt gtcttgggag 4260
170 attggacgac agcgtgggcc ggcccctgtc tcttgatcag atcttgatcc cctgcgccat 4320
172 cagatccttg gcggcaagaa agccatccag tttactttgc agggcttccc aaccttccca 4380
174 gagggcgccc cagctggcaa ttccggttcg cttgctgtcc ataaaaccgc ccagtctagc 4440
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Input Set : **A:\PTO.VSK.txt**
Output Set: **N:\CRF4\03112003\I677584.raw**

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180 ttctacgtgt tccgcttcct ttagcagccc ttgcgccctg agtgcttgcg gcagcgtgaa   4620
182 gctttctctg agctgtaaca gcctgaccgc aacaaacgag aggatcgaga ccatccgctc   4680
184 cagattatcc ggctcctcca tgcgttgcct ctcggctcct gctccggttt tccatgcctt   4740
186 atggaactcc tcgatccgcc agcgatgggt ataaatgtcg atgacgcgca aggcttgggc   4800
188 tagcgactcg accggttcgc cggtcagcaa caaccatttc aacggggtct caccccttggg  4860
190 cgggttaatc tcctcggcca gcaccgcgtt gagcgtgata ttcccctgtt ttagcgtgat   4920
192 gcgcccactg cgcaggctca agctcgcctt gcgggctggt cgattttttac gtttaccgcg   4980
194 tttatccacc acgcccttt gcggaatgct gatctgatag ccacccaact ccggttggtt   5040
196 cttcagatgg tcgatcagat acaacccaga ctctacgtcc ttgcgtgggt gcttggagcg   5100
198 caccacgaag cgctcgttat cgccagcct gtcctgcaga taagcatgaa tatcggcttc    5160
200 gcggtcacag accgcaatca cgttgctcat catgctgccc atgcgtaacc ggctagttgc   5220
202 ggccgctgcc agccatttgc cactctcctt ttcatccgca tcggcaggt catccgggcg    5280
204 catccaccac tcctgatgca gtaatcctac ggtgcggaat gtggtggcct cgagcaagag   5340
206 aacggagtga acccaccatc cgcgggattt atcctgaata gagcccagct tgccaagctc   5400
208 ttcggcgacc tggtggcgat aactcaaaga ggtggtgtcc tcaatggcca gcagttcggg   5460
210 aaactcctga gccaacttga ctgtttgcat ggcgccagcc tttctgatcg cctcggcaga   5520
212 aacgttggga ttgcggtaaa atcggtaagc gccttcctgc atggcttcac taccctctga   5580
214 tgagatggtt attgatttac cagaatattt tgccaattgg cggcgacgt taaccaagcg    5640
216 ggcagtacgg cgaggatcac ccagcgccgc cgaagagaac acagatttag cccagtcggc   5700
218 cgcacgatga agagcagaag ttatcatgaa cgttaccatg ttaggaggtc acatggaagt   5760
220 cagatcctgg aaaacgggaa aggttccgtt caggacgcta cttgtgtata agagtcag     5818
223 <210> SEQ ID NO: 2
224 <211> LENGTH: 476
225 <212> TYPE: PRT
226 <213> ORGANISM: Escherichia coli
228 <400> SEQUENCE: 2
230 Met Ile Thr Ser Ala Leu His Arg Ala Ala Asp Trp Ala Lys Ser Val
231 1               5                   10                  15
233 Phe Ser Ser Ala Ala Leu Gly Asp Pro Arg Arg Thr Ala Arg Leu Val
234             20                  25                  30
236 Asn Val Ala Ala Gln Leu Ala Lys Tyr Ser Gly Lys Ser Ile Thr Ile
237         35                  40                  45
239 Ser Ser Glu Gly Ser Glu Ala Met Gln Glu Gly Ala Tyr Arg Phe Tyr
240     50                  55                  60
242 Arg Asn Pro Asn Val Ser Ala Glu Ala Ile Arg Lys Ala Gly Ala Met
243 65                  70                  75                  80
245 Gln Thr Val Lys Leu Ala Gln Glu Phe Pro Glu Leu Leu Ala Ile Glu
246             85                  90                  95
248 Asp Thr Thr Ser Leu Ser Tyr Arg His Gln Val Ala Glu Glu Leu Gly
249             100                 105                 110
251 Lys Leu Gly Ser Ile Gln Asp Lys Ser Arg Gly Trp Trp Val His Ser
252         115                 120                 125
254 Val Leu Leu Leu Glu Ala Thr Thr Phe Arg Thr Val Gly Leu Leu His
255     130                 135                 140
257 Gln Glu Trp Trp Met Arg Pro Asp Asp Pro Ala Asp Ala Asp Glu Lys
258 145                 150                 155                 160
260 Glu Ser Gly Lys Trp Leu Ala Ala Ala Ala Thr Ser Arg Leu Arg Met
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Input Set : **A:\PTO.VSK.txt**
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```
261                  165                 170                 175
263 Gly Ser Met Met Ser Asn Val Ile Ala Val Cys Asp Arg Glu Ala Asp
264                  180                 185                 190
266 Ile His Ala Tyr Leu Gln Asp Arg Leu Ala His Asn Glu Arg Phe Val
267          195                 200                 205
269 Val Arg Ser Lys His Pro Arg Lys Asp Val Glu Ser Gly Leu Tyr Leu
270      210                 215                 220
272 Ile Asp His Leu Lys Asn Gln Pro Glu Leu Gly Gly Tyr Gln Ile Ser
273 225                  230                 235                 240
275 Ile Pro Gln Lys Gly Val Val Asp Lys Arg Gly Lys Arg Lys Asn Arg
276                  245                 250                 255
278 Pro Ala Arg Lys Ala Ser Leu Ser Leu Arg Ser Gly Arg Ile Thr Leu
279          260                 265                 270
281 Lys Gln Gly Asn Ile Thr Leu Asn Ala Val Leu Ala Glu Glu Ile Asn
282          275                 280                 285
284 Pro Pro Lys Gly Glu Thr Pro Leu Lys Trp Leu Leu Leu Thr Gly Glu
285      290                 295                 300
287 Pro Val Glu Ser Leu Ala Gln Ala Leu Arg Val Ile Asp Ile Tyr Thr
288 305                  310                 315                 320
290 His Arg Trp Arg Ile Glu Glu Phe His Lys Ala Trp Lys Thr Gly Ala
291                  325                 330                 335
293 Gly Ala Glu Arg Gln Arg Met Glu Glu Pro Asp Asn Leu Glu Arg Met
294          340                 345                 350
296 Val Ser Ile Leu Ser Phe Val Ala Val Arg Leu Leu Gln Leu Arg Glu
297          355                 360                 365
299 Ser Phe Thr Leu Pro Gln Ala Leu Arg Ala Gln Gly Leu Leu Lys Glu
300      370                 375                 380
302 Ala Glu His Val Glu Ser Gln Ser Ala Glu Thr Val Leu Thr Pro Asp
303 385                  390                 395                 400
305 Glu Cys Gln Leu Leu Gly Tyr Leu Asp Lys Gly Lys Arg Lys Arg Lys
306                  405                 410                 415
308 Glu Lys Ala Gly Ser Leu Gln Trp Ala Tyr Met Ala Ile Ala Arg Leu
309          420                 425                 430
311 Gly Gly Phe Met Asp Ser Lys Arg Thr Gly Ile Ala Ser Trp Gly Ala
312          435                 440                 445
314 Leu Trp Glu Gly Trp Glu Ala Leu Gln Ser Lys Leu Asp Gly Phe Leu
315      450                 455                 460
317 Ala Ala Lys Asp Leu Met Ala Gln Gly Ile Lys Ile
318 465                  470                 475
320 <210> SEQ ID NO: 3
321 <211> LENGTH: 30
322 <212> TYPE: DNA
323 <213> ORGANISM: Artificial sequence
325 <220> FEATURE:
326 <223> OTHER INFORMATION: Defined sequence kernel
328 <220> FEATURE:
329 <221> NAME/KEY: misc_feature
330 <222> LOCATION: (1)..(30)
331 <223> OTHER INFORMATION: n is A, T, G, or C
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Input Set : **A:\PTO.VSK.txt**
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```
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  337 <210> SEQ ID NO: 4
  338 <211> LENGTH: 30
  339 <212> TYPE: DNA
  340 <213> ORGANISM: Artificial sequence
  342 <220> FEATURE:
  343 <223> OTHER INFORMATION: Defined sequence kernel
  345 <220> FEATURE:
  346 <221> NAME/KEY: misc_feature
  347 <222> LOCATION: (1)..(30)
  348 <223> OTHER INFORMATION: n is A, T, G, or C
  350 <400> SEQUENCE: 4
W--> 351 nnmnnmnnmn nmnnmnnmnn mnnmnnmnnm                              30
  354 <210> SEQ ID NO: 5
  355 <211> LENGTH: 5
  356 <212> TYPE: PRT
  357 <213> ORGANISM: Artificial sequence
  359 <220> FEATURE:
  360 <223> OTHER INFORMATION: Antibody spacer peptide. The entire peptide sequence can be
  361       repeated more than one time
  363 <400> SEQUENCE: 5
  365 Gly Gly Gly Gly Ser
  366 1               5
  368 <210> SEQ ID NO: 6
  369 <211> LENGTH: 14
  370 <212> TYPE: DNA
  371 <213> ORGANISM: Artificial sequence
  373 <220> FEATURE:
  374 <223> OTHER INFORMATION: Tetradecanucleotide d
  376 <400> SEQUENCE: 6
  377 catgccatgg catg                                               14
  380 <210> SEQ ID NO: 7
  381 <211> LENGTH: 21
  382 <212> TYPE: DNA
  383 <213> ORGANISM: Artificial sequence
  385 <220> FEATURE:
  386 <223> OTHER INFORMATION: 21-mer d
  388 <400> SEQUENCE: 7
  389 aaattgtgca catcctgcag c                                       21
  392 <210> SEQ ID NO: 8
  393 <211> LENGTH: 12
  394 <212> TYPE: DNA
  395 <213> ORGANISM: Artificial sequence
  397 <220> FEATURE:
  398 <223> OTHER INFORMATION: 12-mer target DNA
  400 <400> SEQUENCE: 8
  401 agcctagctg aa                                                 12
  404 <210> SEQ ID NO: 9
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RAW SEQUENCE LISTING ERROR SUMMARY     DATE: 03/11/2003
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Input Set : **A:\PTO.VSK.txt**
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<u>Please Note:</u>
Use of n and/or Xaa have been detected in the Sequence Listing. Please review the
Sequence Listing to ensure that a corresponding explanation is presented in the <220>
to <223> fields of each sequence which presents at least one n or Xaa.


Seq#:3; N Pos. 1,2,4,5,7,8,10,11,13,14,16,17,19,20,22,23,25,26,28,29
Seq#:4; N Pos. 1,2,4,5,7,8,10,11,13,14,16,17,19,20,22,23,25,26,28,29
Seq#:10; Xaa Pos. 3
Seq#:12; Xaa Pos. 2
Seq#:13; N Pos. 6,7,8,9,10
Seq#:14; N Pos. 1,2,3,4,5,6,7,8,9,10,21,22,23,24,25,26,27,28,29,30,31,32,33
Seq#:14; N Pos. 34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53
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Seq#:14; N Pos. 110,111,112,113,114,115,116,117,118,119,120,124,125,126,127
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Seq#:15; N Pos. 211,212,213,214,215
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Seq#:16; N Pos. 44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63
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Seq#:16; N Pos. 84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101,102
Seq#:16; N Pos. 103,104,105,106,107,108,109,110,111,112,113,114,115,116,117
Seq#:16; N Pos. 118,119,120,121,122,123
Seq#:17; N Pos. 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41
Seq#:17; N Pos. 42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61
Seq#:17; N Pos. 62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81
Seq#:17; N Pos. 82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100
Seq#:17; N Pos. 101,102,103,104,105,106,107,108,109,110,111,112,113,114,115
Seq#:17; N Pos. 116,117,118,119,120,121

**VERIFICATION SUMMARY**                        DATE: 03/11/2003
PATENT APPLICATION:  **US/09/677,584**          TIME: 10:54:54

Input Set : **A:\PTO.VSK.txt**
Output Set: **N:\CRF4\03112003\I677584.raw**

```
L:4 M:283 W: Missing Blank Line separator, <120> field identifier
L:9 M:270 C: Current Application Number differs, Replaced Current Application Number
L:10 M:271 C: Current Filing Date differs, Replaced Current Filing Date
L:334 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:3 after pos.:0
L:351 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:4 after pos.:0
L:431 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:10 after pos.:0
L:462 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:12 after pos.:0
L:479 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:13 after pos.:0
L:511 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:14 after pos.:0
M:341 Repeated in SeqNo=14
L:549 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:15 after pos.:0
M:341 Repeated in SeqNo=15
L:577 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:16 after pos.:0
M:341 Repeated in SeqNo=16
L:603 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:17 after pos.:0·
M:341 Repeated in SeqNo=17
```